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A striking feature of vertebrate embryos is the bilateral symmetry of their exterior body plan, that is also reflected in the development of limb buds in pairs. Mendelian inheritance analyses now provide an unexpected link between two important topics of developmental biology, namely limb development and left–right asymmetry. Among teleost killifishes (Cyprinodontidae) some species completely lack a pair of pelvic fins (e.g., *Aphanius apodus* Gervais 1853), and they are homozygote for this character. To study the genetics of fin induction this species was crossed with closely related killifishes that possess pelvic fins (*A. iberus* Cuv. and Val. 1846). Astonishingly, a constant proportion of the hybrid offspring developed only a single pelvic fin. Also, in natural populations of *A. iberus*, specimens can be found having single pelvic fins on either the right or left side. Selective breeding of these single-finned individuals has revealed that fin laterality is not random but genetically controlled. The evidence for an independent genetic control of left- versus right-limb induction suggests the remarkable hypothesis that the development of vertebrate limbs in pairs is not a simple default state, as might have been be supposed by the idea of establishing bilateral mirror image appendages in pairs based on a single signaling source. Instead, a bilateral balanced genetic stabilization seems to be necessary to initiate every individual left and right limb bud. Therefore there must exist an unexpected mechanism for assigning left and right identity to seemingly identical structures (such as limbs), thus revealing a cryptic underlying polarity.

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Limb heterochrony in a marsupial, *M. domestica*

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Heterochrony, or a shift in developmental timing, is an important source for evolutionary change. Here, we utilize *Monodelphis domestica* (Metatheria), an opossum, as a model to investigate the developmental origins of limb heterochrony. *M. domestica* neonates show significant acceleration of forelimb (FL) development relative to the hindlimbs (HL) when compared to other non-metatherian amniotes. When the HLs of *M. domestica* embryos begin outgrowth, the FLs are well-defined buds, indicating that heterochrony arises very early, before outgrowth of either limb. There are at least three potential mechanisms by which this heterochrony might be produced. FL outgrowth could be initiated earlier than HL outgrowth by virtue of earlier genetic signaling to the FL field or later signaling to the HL field. Alternatively, a slowing of axis extension and cell proliferation in the primitive streak could limit tissue availability in the posterior lateral plate mesoderm (LPM), so that the field of cells that will eventually give rise to the HL field are not yet present when the FL begins outgrowth. Similarly, at the time of FL outgrowth initiation, the axis may not have extended far enough for the HL field to have been specified. That is, there may be LPM present that will eventually be specified as HL, but it does not yet have an anterior–posterior positional identity. In this study we provide in situ hybridization data to distinguish these mechanisms. We detail the expression patterns of *tbx4*, *tbx5*, and *fgf10*. Results suggest that slowed axis extension may limit tissue availability from the streak and delay the initiation of HL outgrowth.

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Comparative development of mammalian and alligator metapodial growth plate formation

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Mammalian metapodials, unlike most long bones, form only a single growth plate. We have shown that the mouse metatarsal growth plate displays a peak of chondrocyte proliferation corresponding to the 'resting zone' that contrasts with the more diffuse pattern at the non-physis end undergoing 'direct ossification'. To determine if this particular mode of ossification and growth is unique to mammalian metapodials, we traced the development of the growth plate in the alligator (*Alligator mississippiensis*). We find that, unlike mammals, growth plates form at both ends of the alligator metapodial and are maintained in the subadult. Prior to growth plate formation, embryonic alligators differ from mice in showing poor columnar organization and gradual transition from columnar to hypertrophic chondrocytes, though once growth plates are established these parameters are largely similar. Immunohistochemistry reveals that key factors that regulate growth plate formation in mammals (Ihh and PTHrP) are also expressed in alligators. PCNA results show that during growth plate formation alligators exhibit the same proliferative peak in their presumptive resting zones as mice, indicating that this is potentially a key event in the development of a growth plate. The broad commonalities in mouse and alligator growth plates indicate that direct ossification of one epiphysis and reliance on a single growth plate are a novel adaptation in mammalian metapodials. Our analysis also shows that the anatomical locations of growth plates have been modified during vertebrate evolution. Supported by NSF and NIH.

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Effect of a single dose of ethanol on developing peripheral nerve of chick embryos

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Fetal alcohol syndrome (FAS), a condition occurring in some children of mothers who have consumed alcohol during

pregnancy, is characterized by physical and mental growth retardation and craniofacial anomalies. Though motor and sensory derangements and neurobehavioral deficits have been reported in FAS, no specific changes in the brain and spinal cord that could conclusively explain these neuronal defects have been reported. The aim of this study was to investigate effects of a single high dose of ethanol during early gestation on the peripheral nerve. Chick embryos were exposed to 5%, 10% and 15% ethanol, and general growth and development, and peripheral nerve of ethanol exposed embryos was examined following full gestation. There was dose-dependent reduction in body parameters in ethanol exposed chicks, and significant prenatal mortality was observed in embryos exposed to 15% ethanol. In majority of embryos that survived exposure to 15% ethanol, features of myelin degeneration were observed. No myelin degeneration was observed in embryos exposed to 5% and 10% ethanol, and in the control groups, thus demonstrating the direct toxic effects of a single high dose of ethanol on developing embryos in general and peripheral nerve in particular. This offers a possible explanation for some of the neuronal disorders observed in FAS, particularly in the light of a recent report describing significant impairment in nerve conduction velocity and amplitude changes in peripheral nerves in children exposed to alcohol during gestation.

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Divergent axon growth patterns among arthropods coincide with changes in netrin expression patterns

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Despite the presence of homologous neuroblasts and neurons in crustaceans and insects, we have observed differences in ventral nerve cord formation among arthropods. In both long and short germ insects and in some crustaceans, commissural axon tracts are established prior to the longitudinals. However, in several branchiopod crustaceans, such as the brine shrimp *Artemia franciscana*, we have noted that the longitudinal axon tracts are pioneered prior to commissural axon growth. To determine if changes in the expression of axon guidance molecules may be responsible for such differences, we cloned the *Artemia franciscana* netrin (afrnet) gene. An antibody to the afrNet protein was generated and uncovered a posterior domain of afrNet expression at the time when longitudinal axons are established. *Drosophila*-like midline expression of afrNet is detected at a later timepoint when commissural axons are extending toward the midline. Our afrNet antibody was found to cross-react to Netrin proteins in other insect and crustacean species, a discovery that has allowed us to study Netrin expression patterns across arthropods. Our findings indicate that the changes in Netrin expression patterns observed in Artemia are conserved among branchiopods. However, in short germ insects and malacostracan crustaceans, axon guidance and Netrin expression is more comparable to that of *Drosophila*.

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Selection on neurodevelopmental actions of thyroid hormone underlies the conservation of thyroid hormone receptor genes in *Necturus maculosus*

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The mudpuppy (*Necturus maculosus*) is an obligate pedomorphic amphibian. In metamorphosing amphibians thyroid hormone (T_3) induces tissue transformations by binding to nuclear T_3 receptors (TRs). Earlier work showed that T_3 treatment of adult *Necturus* failed to induce metamorphosis. However, recent findings show that *Necturus* TR genes encode fully functional TR proteins. Thus, natural selection has maintained the structure and function of *Necturus* TRs, suggesting that they play critical, as yet undiscovered roles. We treated larval *Necturus* with T_3 (200 nM) in the aquarium water and analyzed external morphology, gene expression in brain and gill, and neural cell proliferation. While T_3 treatment caused whole organism metabolic effects it failed to induce external morphological changes, consistent with earlier work in adults. We analyzed by RT-PCR the expression of TRβ, a gene known to be autoinduced in metamorphosing species. TRβ mRNA was strongly induced in *Necturus* larval brain, but not in gill. T_3 treatment caused a robust increase in the number of mitotic cells in *Necturus* larval brain. Our findings support the hypothesis that functional TRs have been maintained by natural selection for essential neurodevelopmental actions of T_3. By contrast, larval organs such as the gill have lost responsiveness to T_3, perhaps due to the failure to upregulate TRs to a sufficient level to support T_3 action (supported by NSF grant IBN0235401 to R.J.D.).

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Sex pheromone in dioecious nematode: Its production and perception pathway

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Two different mating systems are adopted by *Caenorhabditis* species, dioecy and androdioecy. For dioecious species, finding a mating partner is an obligatory process for survival. We have identified a sex-specific attractant produced by dioecious *Caenorhabditis remanei* females to